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Ketamine Prevents Learning Impairment When Administered Immediately after Status Epilepticus Onset

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Permanent cognitive impairment is common following status epilepticus (SE) in both humans and animals. We examined the effect of the NMDA antagonist ketamine administered after SE onset on two forms of associative learning in the rat: conditioned taste aversion and fear-conditioned analgesia. Following the onset of lithium/pilocarpine-induced SE, rats were administered either ketamine (100 mg/kg) or acepromazine (25 mg/kg). Acepromazine-treated animals show marked deficits in both learning measures at 1 month after SE. In contrast, ketamine-treated and nonepileptic control animals did not differ in performance for either task. Although studies have shown that ketamine is ineffective at controlling electrographic seizures early in SE, these results are consistent with previous studies showing that ketamine can preserve learning proficiency if administered shortly after seizure onset. As a clinically available drug, ketamine may prove useful in the treatment of SE when combined with conventional antiepileptic strategies. © 2001 Elsevier Science

Key Words: **ketamine; status epilepticus; pilocarpine; conditioned taste aversion; fear conditioning; learning; rat.**

INTRODUCTION

Status epilepticus (SE) is among the most severe forms of epilepsy and is regarded as a medical emergency associated with considerable morbidity and mortality (1). Permanent cognitive impairment is one of many neurologic deficits identified with both human and experimental SE (2–4). This is thought to be due in part to the adverse metabolic and physiologic effects of generalized convulsive SE or to widespread neuronal damage caused by excessive electrical activity in the brain (2). Either case emphasizes the importance of early and effective treatment for improving clinical outcome.

The pilocarpine (PILO) model of SE has been widely used to study acute SE as well as the pathogenesis of chronic epilepsy (5). Although the mortality rate associated with PILO-induced SE is high (~80%) (6), with appropriate treatment rats can survive and develop many of the pathologic sequelae associated with human epilepsy, such as axonal sprouting (7), interneuron loss (8), spontaneous recurrent seizures (9), and interictal discharges (5). Moreover, transient episodes of PILO-induced SE (<1 h) can produce marked learning impairment for tests of spatial and associative learning (10, 11). However, if the noncompetitive NMDA antagonist ketamine is administered within minutes of seizure onset, spatial learning proficiency is spared (12, 13). As a clinically available drug, ketamine has proven useful in the treatment of refractory SE (14) and is neuroprotective in the PILO model (15).

In the present study, we examined the effect of ketamine administered after SE onset on two forms of associative learning in the rat: conditioned taste aversion (CTA) and fear-conditioned analgesia (FCA). In the CTA paradigm, an animal learns to associate a

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novel taste cue with drug-induced malaise so that a future exposure to the taste stimulus becomes aversive. This form of learning is extremely rapid, usually acquired with a single pairing, and appears resistant to long taste–illness delays (16). On the other hand, FCA is not a form of learning per se, but rather a physiologic response that occurs as a result of learning. For example, if an animal is placed in a novel environment and then delivered an electric shock, the background or contextual cues of that environment become associated with the stressful event such that a subsequent exposure can evoke innate defensive reactions such as immobility and analgesia (17). Like conventional learning paradigms, the development of FCA requires an animal to form an association between stimulus cues and to be able to retrieve that representation to engage in appropriate behavior. Using these two paradigms, the main goals of this study were: (1) to complement existing behavioral data by further evaluating the role of ketamine in preventing cognitive impairment following PILO-induced SE, and (2) to compare the behavioral effect of ketamine with that of acepromazine, an atypical neuroleptic that suppresses behavioral seizures and prevents cardiac instability during SE (18).

METHOD

Subjects and Drug Administration

After approval by the Laurentian University research ethics committee and in accordance with Canadian Animal Care Council guidelines, male Wistar strain rats (80–90 days of age; Charles River Breeders, Quebec) were administered lithium chloride (3 mEq/kg, sc) followed 4 h later by PILO (30 mg/kg, sc). Lithium pretreatment has been shown to reduce the convulsant dose of PILO more than 10-fold (6). Following the onset of stage 3–5 motor seizures (stage 3, forelimb clonus; stage 4, forelimb clonus with rearing; stage 5, forelimb clonus, rearing and loss of posture; see Ref. 19), animals received a single injection of either ketamine (100 mg/kg, sc; $N = 15$) or acepromazine (25 mg/kg, sc; $N = 15$). An additional group of age-matched animals ($N = 13$) served as a nonepileptic reference group. For ethical reasons, a saline/PILO group was not included in the study because vehicle-treated rats invariably die within 24 h. Behavioral testing began 30 days after SE.

Conditioned Taste Aversion

With food readily available, animals were housed in single cages and water deprived for 24 h. All rats were then habituated to receive a daily ration of water during a 20-min interval. After 5 days, the rats were presented with a 10% sucrose solution for 20 min followed 30 min later by a peripheral (ip) injection of 10 mL/kg 0.15 M lithium chloride solution. A retrieval test was conducted after a 2-day recovery period (during which water was given for 20 min per day) where the sucrose was again presented for 20 min. Taste aversion scores were computed as the ratio of sucrose consumed on test day (mL) to sucrose consumed on pairing day.

Fear-Conditioned Analgesia

Prior to CTA, pain responses were measured using the hotplate procedure (20). The apparatus consisted of a cubical Plexiglas chamber that confined the rat to a metal plate heated to a temperature of 55 ± 1°C. The time elapsing from the contact of the animal with the plate to licking both hind paws was recorded as the hotplate response latency and used as an inference of pain threshold. Baseline pain thresholds were measured and averaged over a 3-day block.

The fear conditioning procedure has been described in detail elsewhere (21). Briefly, each animal was placed in a Plexiglas chamber for 2 min. This baseline period allows the rat to explore and acquire various contextual (spatial) cues of the training apparatus. At the end of 2 min, a single footshock (0.5 mA for 1 s) was delivered through steel bars composing the chamber floor. The animal was left in the chamber for 60 s after the footshock and then returned to its home cage. After five daily conditioning trials, each animal was returned to the conditioning chamber (Day 6) for a 5-min period (no shock delivered) and then placed on the hotplate. If the rat "successfully" associated the chamber environment with aversive stimulation (i.e., if learning has occurred), posttraining exposure to the chamber would presumably evoke innate defensive reactions (i.e., memory), such as an increase in pain threshold. FCA was defined as the relative change in hotplate response latency following the conditioning trials compared with baseline: FCA = (postconditioning − baseline)/baseline.

Histology

At the conclusion of the behavioral testing, brains were removed and postfixed in EFA (ethanol/forma-

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lin/acetic acid) solution for 72 h. The tissue was then embedded in parrafin blocks, sectioned at 10 μm, and stained with toludine blue O for microscopic analysis of the hippocampus, amygdala, and entorhinal cortex. These brain regions play a key role in the development of PILO seizures and have been shown to exhibit marked neuronal loss following SE (10, 11).

Data Analysis

Between-group differences in the behavioral measures were examined using one-way analyses of variance (ANOVAs) with post hoc Tukey tests. Group differences were considered statistically significant at $P < 0.05$.

RESULTS

PILO-Induced Seizures

PILO-induced motor seizures began with mild body tremor and salivation that progressed to bouts of face, neck, and forelimb clonus with rearing and loss of posture. The mean seizure onset latency for all subjects was 31.37 min (±3.41 min). There was no significant difference in seizure latency between treatment groups. All animals were visually monitored for 30–60 min following seizure onset. Behavioral seizures were completely suppressed within 5 min of ketamine or acepromazine injection and did not recur during the remaining observation period. Observations from previous studies in our laboratory have indicated that behavioral seizures are often completely suppressed following these treatments and do not recur until chronic seizures develop (unpublished data).

CTA Learning

An ANOVA indicated a significant group difference in the ratio of sucrose consumed on test day to sucrose consumed on pairing day [$F(2, 42) = 27.45$, $P < 0.01$]. Post hoc analysis revealed the source of the difference to be a greater sucrose intake on test day compared with pairing day for acepromazine-treated animals relative to ketamine-treated and control animals, which did not differ from each other at $P < 0.05$ (Fig. 1A).

Fear-Conditioned Analgesia

Baseline hotplate trials did not show significant within-group differences for any of the treatments (data not shown). However, there was a significant difference in hotplate latencies between groups [$F(2, 22) = 13.28$, $P < 0.01$]. Acepromazine-treated animals displayed a slight elevation in response latency compared with the ketamine and reference groups (Fig. 1B).

A significant difference in FCA between the treatment groups [$F(2, 22) = 17.39$, $P < 0.01$] was found. Post hoc analysis indicated that acepromazine-treated animals did not show a relative increase in hotplate latency following the conditioning trials compared with the remaining groups (Fig. 1C). Although not quantified in the present experiment, both ketamine-treated and control animals also began to display defensive immobility (i.e., freezing) in response to the conditioning environment as early as the second day of conditioning, whereas acepromazine-treated animals showed little evidence of freezing throughout the trials. Freezing behavior is characterized by a complete cessation of movement except that required for respiration and is commonly used as an inference of associative learning in fear conditioning paradigms (22).

Histological Analysis

At approximately 50 days post-SE, qualitative histologic examination (Fig. 2) revealed marked neuronal loss in CA1 of the hippocampal formation for both treatment groups, with acepromazine-treated animals showing near-complete destruction of the CA1 pyramidal cells layer. Neuron loss and gliosis were also visually evident in the amygdala (basolateral nucleus) of both groups. Qualitative changes in neuronal density were observed for the amygdala–hippocampal transition area, although cell loss appeared more extensive in ketamine-treated animals. This selective cell loss has been previously reported (10, 11) and is thought to prevent the spread of chronic seizures from the limbic system to motor structures. Prominent cell damage was present in the entorhinal cortex of both treatment groups. Such necrosis often extends into the neighboring temporal and piriform cortices as well as the corticoamygdala (not shown). The micrographs presented in Fig. 2 were taken from the left hemisphere. However, past studies have shown that pathologic changes associated with PILO-induced SE are bilateral and statistically similar in magnitude (6, 10–13).

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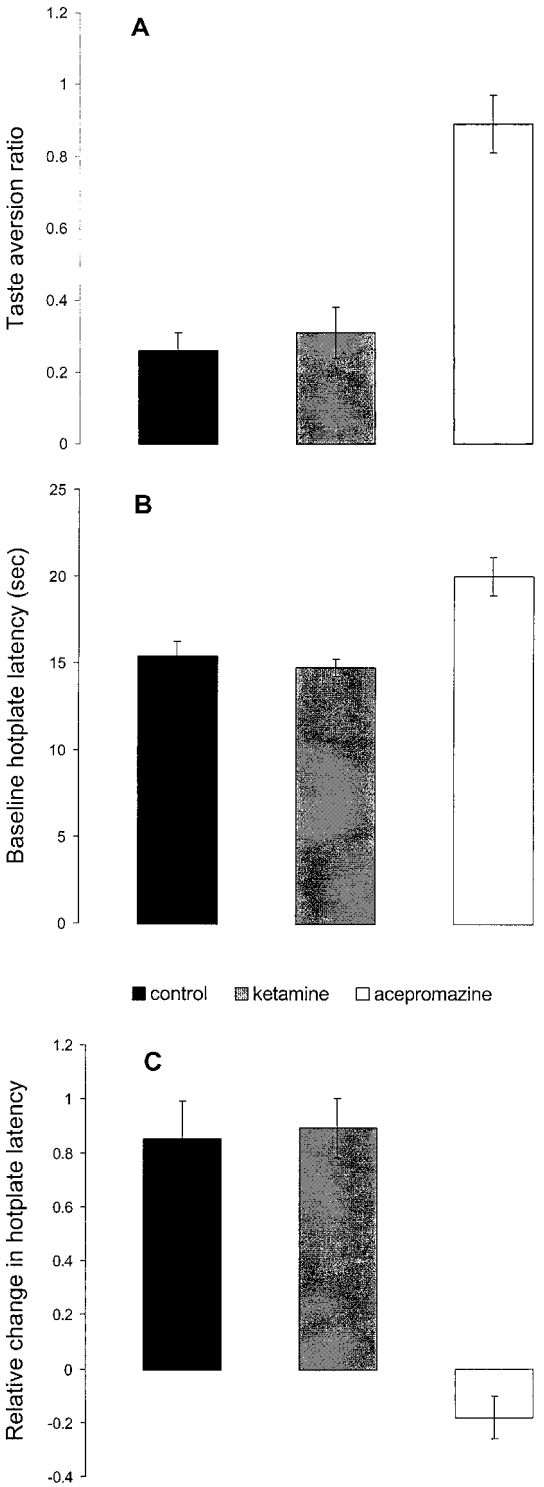

FIG. 1. Effects of acepromazine and ketamine administered after PILO-induced SE on behavioral measures. (A) CTA formation was measured as the ratio of sucrose consumed on test day to sucrose consumed on pairing day. Each bar represents the mean (±SEM) CTA ratio. (B) Baseline hotplate response latencies for control and treatment groups were measured prior to the start of conditioning trials. Response latencies were calculated as the elapsed time (s) from the animal being placed on the heated plate to licking each hindpaw. This response was used as an inference of pain threshold. (C) Changes in hotplate response latencies were measured after five daily fear conditioning trials (see text for details). Animals were returned to the conditioning chamber for 5 min and then moved to the hotplate apparatus. Data represent change in hotplate latency following the 5-min exposure period relative to baseline (each animal was used as its own control).

DISCUSSION

The major finding of this study was that associative learning deficits characteristic of PILO-induced SE were prevented when ketamine, a clinically available NMDA antagonist, was administered following seizure onset. In the first experiment, acepromazine treatment resulted in a marked attenuation of CTA, indicated by an absence of an aversion to sucrose 2 days after pairing. In contrast, animals treated with ketamine reached a level of aversion equivalent to that of nonepileptic control animals. The results of experiment 2 suggest that the development of FCA during a fear conditioning paradigm was also impaired following acepromazine treatment compared with remaining groups. Similar findings have been reported with ketamine and maze performance in rats (12, 13). However, our results indicate that the protective effect of ketamine on cognitive function is not exclusive to spatial learning but extends to other forms of associative learning despite the presence of forebrain cell loss.

While the behavioral paradigms used in this study may not be considered conventional tests of cognitive function in the epilepsy literature, they represent fundamental associative processes that are operative in other types of learning tasks. CTA is characterized by long-term avoidance behavior that is established after an individual learns to associate a novel gustatory stimulus with the consequences of its ingestion. The CTA thus serves as a defense mechanism protecting animals, or humans, against repeated consumption of potentially toxic substances (16). Previous studies have described a marked attenuation of CTA formation following PILO-induced SE treated with acepromazine (11, 23). A possible explanation for this impairment lies in the extent of brain damage that follows SE. Neuroanatomic studies have indicated that forebrain areas such as temporal cortex and amygdala are critical for CTA formation. These structures occupy the terminal end of a sensory pathway that originates in cranial nerves VII, IX, and X (facial,

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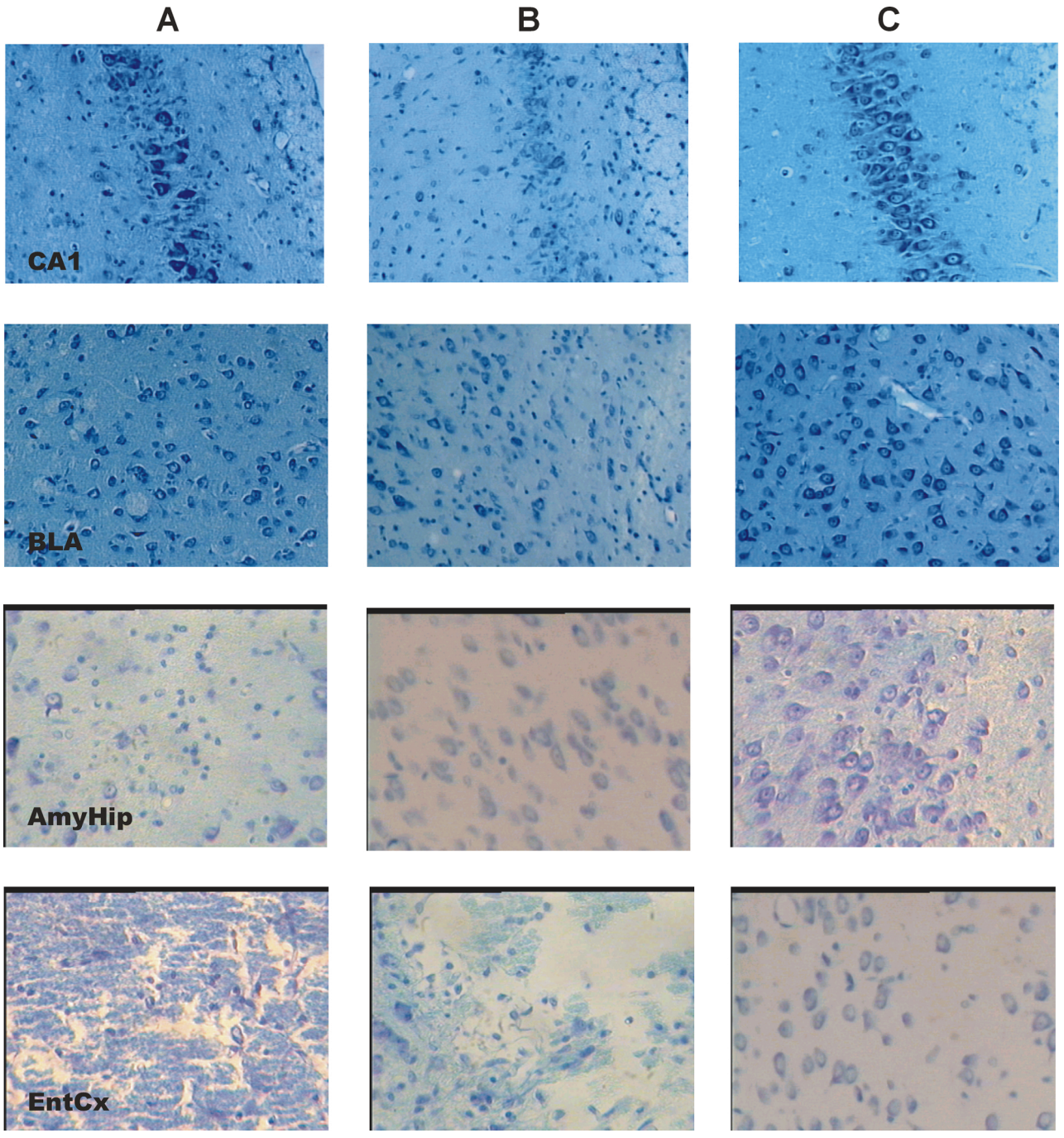

FIG. 2. Comparison of transverse sections taken from the forebrain of treatment and control groups at 50 days post-SE: (A) ketamine, (B) acepromazine, (C) nonepileptic controls. Both acepromazine- and ketamine-treated animals showed qualitative neuronal loss in all sections examined although the extent of cell loss appeared to differ in some structures, i.e., amygdala–hippocampal area (Nissl staining, magnification 400x). CA1, layer CA1 of the hippocampus; BLA, basolateral amygdala; AmyHip, amygdala–hippocampal transition area; EntCx, entorhinal cortex.

glossopharnygeal, and vagus, respectively) and ascends through the midbrain and thalamus (24). In the PILO model, the amygdala and temporal regions normally show extensive cell loss 30–50 days after SE (Fig. 2) whereas more caudal (mesencephalic) structures do not (25). Although the irreversibility of sei-

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zure-induced brain damage makes it difficult to determine whether the absence of CTA was due to impairment of CTA acquisition or impairment of CTA retrieval, specific damage to the basolateral amygdala as well as gustatory areas of temporal cortex (10, 25) would likely prevent acquisition (24).

Bilateral damage to specific regions of the amygdala, such as the central and basolateral nuclei, attenuates analgesic responses in aversive conditioning paradigms (26). Central amygdala lesions are thought to antagonize the expression of FCA produced by aversive environments by disrupting descending connections with the midbrain periaqueductal gray (26). Given that the central amygdala and periaqueductal show no obvious cell loss following SE (10, 25), basolateral and possibly hippocampal, damage may account for the observed deficits in FCA for acepromazine-treated animals (Fig. 2). Posttraining lesions restricted to the basolateral amygdala often result in a complete loss of fear-conditioned responses (27). However, in the present study basolateral damage would have occurred prior to training, thus interfering with the associative aspects of the learning task, i.e., the ability to form an association between local chamber cues and an aversive stimulus (28). Alternatively, the deficit in FCA observed after acepromazine treatment could relate to abnormal pain sensation. Because these animals displayed significantly longer hotplate latencies during baseline testing, it is possible that the FCA deficit may be the result of a difference in perceived shock intensity during the conditioning trials rather than an actual learning impairment.

Previous studies have shown that the degree of neuronal damage produced by an episode of PILO-induced SE does not differ between acepromazine and ketamine treatments (12, 13). In electrogenic models of SE, ketamine is ineffective at controlling seizures when administered during the early phase of SE (14, 29). NMDA antagonists such as ketamine and MK-801 are use-dependent; repeated seizures must occur before the NMDA ionophore becomes activated and provides a suitable environment for antagonists (30). When ketamine is administered 15 min after seizure onset (followed by a combination of atropine, diazepam, and pentobarbital) it can provide near-complete neuroprotection in the limbic forebrain (15). While behavioral seizures may have been suppressed, ketamine likely failed to limit brain damage in past studies because it was administered too early in SE and was not followed by conventional antiepileptic drugs to terminate electrographic seizures. Despite this, the finding that ketamine can protect against cognitive impairment, even when administered immediately after seizure onset, is clinically significant. Acepromazine is a dopamine receptor antagonist and although it is effective at suppressing behavioral seizures and preventing respiratory arrest during SE (18), it has no effect on electrographic seizures (unpublished data). Acepromazine was originally developed as an antipsychotic but was dropped from clinical practice during the 1950's because it did not produce the desired effects seen with other phenothiazine derivatives (although it is still used in veterinary medicine as an adjunct to surgical anesthesia).

Ketamine offers an additional advantage in the treatment of PILO seizures. Although animals show significant forebrain pathology following SE (12, 13), they often do not enter a chronic phase of spontaneous recurrent seizures (3; unpublished observations). The absence of chronic seizures may result from subtle differences in cell loss in certain limbic structures, such as the amygdala–hippocampal transition area (Fig. 2) (12, 13) that prevents focal limbic seizures from propagating and becoming secondarily generalized. This leaves open the possibility that for certain types of learning there may be little functional significance to the seizure-induced cell loss since there is no overall difference in the amount of brain damage between the two treatments. Rather, a chronic seizure state perturbs ongoing brain activity, which contributes more to cognitive deficits than a single episode of SE.

In summary, the present data indicate that the NMDA antagonist ketamine can prevent learning impairment when administered immediately after the onset of PILO-induced SE. The protective effects of ketamine on cognitive function and neuronal density (15) suggest that it may be of value as adjunctive therapy in the clinical treatment of SE. However, before clinical studies can be considered, further experiments are necessary in view of data indicating that delayed ketamine treatment (~30 min) does not prevent cognitive impairment (12). In addition, dose–response studies may be required given that ketamine is a potent anesthetic and high doses would likely cause respiratory trauma.

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